UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION
WASHINGTON 25, D. C.

PREPARATION OF K ANTIGEN

AA - Materials

1. Agar U.S.P., selected for culture media.
2. Beef, lean; fat trimmed off.
3. Crystal violet, extra pure, certified, not less than 85 percent dye content.
4. Glycerin U.S.P. (95 percent).
5. Liquor Formaldehyde U.S.P. (37 percent CH_2O).
6. Peptone (Armour's).
7. Phenol red indicator solution.
8. Sodium chloride U.S.P.
9. Sodium hydroxide
   (a) 0.5 N solution for titration (phenol-red indicator)
   (b) 2.0 N solution for adjustment
10. Sulfur, colloidal (Eimer and Amend).
11. Potassium phosphate, monobasic (Sorensen) KH_2PO_4
12. Water, distilled.

BB - Preparation of Culture Media

1. Beef-infusion: 500 grams of ground beef is mixed with 1000 cc. of water. After one hour at room temperature the mixture is heated at 55° to 60° C., and held, with occasional stirring, for two hours. It is then strained through cheese-cloth, and the residue is pressed. The extract is brought just to the boiling point and immediately filtered through paper.

2. Beef-infusion broth: To 1000 cc. of beef infusion are added peptone, 20 grams, and sodium chloride, 5 grams. The mixture is boiled 15 minutes, adjusted to pH 7.0 and filtered through paper until clear. After distribution into tubes, the broth is sterilized in an autoclave under 15 pounds steam pressure for 15 minutes.

3. Special beef-infusion agar: To 1000 cc. of beef infusion are added 5 grams of sodium chloride, 20 grams of peptone, and 30 grams of agar. The mixture is heated in an autoclave under 15 pounds steam pressure until the agar is dissolved, 30 minutes or more. The reaction is adjusted to pH 7.0, and one gram of colloidal sulfur suspended in 20 cc. of glycerin is added. The sulfur may be suspended in the glycerin by heating gently on a steam bath or electric hot plate, shaking frequently until a uniform suspension is obtained. After adding the sulfur and glycerin, the culture medium should be shaken vigorously and then distributed in 100 cc. portions into 300 cc. Erlenmeyer flasks, shaking frequently while the flasks are being filled.

This culture medium is not filtered. Final sterilization is effected in the autoclave under 15 pounds steam pressure for 30 minutes. After this final sterilization, the flasks are shaken until the culture medium is as uniform in appearance as possible, and are then allowed to stand at room temperature until the agar solidifies. The medium is allowed to become thoroughly firm before being seeded.

CC - Maintenance of Stock Cultures

Approved stock cultures of Salmonella pullorum are maintained on plain beef-infusion agar containing one percent peptone and 2 percent agar, and adjusted to pH 7.0.

DD - Seeding for antigen production

Tubes of beef infusion broth are seeded from agar stock cultures not more than one month old. After 24 hours incubation, these cultures are used to seed the flasks of special beef-infusion agar, using one cc. of culture for each flask, and flowing it over the surface as uniformly as possible. Approximately an equal number of flasks should be seeded with each of the strains. The flasks are capped with tinfoil and incubated at 37.5° C. for 6 days. They should show a smooth, dense, and uniform growth.

EE - Harvesting the Growth

To each flask of acceptable growth is added 40 cc. of formaldehyde-salt solution (Liquor Formaldehyde one percent, sodium chloride 0.85 percent). After a 15-20 minute soaking, the growth is detached from the agar with a wing-tipped rubber policeman, the flask twirled a few times, and the liquid contents decanted into a suitable receiving vessel. The flasks are then serially rinsed with a total quantity of about 60 cc. of formaldehyde-salt solution to each 10 flasks, the rinsings being finally transferred to the original receiving vessel. The suspension is filtered through a layer of cotton-wool in a Buchner funnel with the aid of slight suction. Bacterial masses remaining at the bottom of the receiving vessel are macerated by vigorous twirling of the vessel. Sufficient formaldehyde-salt solution is then added for free passage of the remaining suspension through the cotton pad. It may be necessary to replace the cotton pad from time to time, depending on the quantity of antigen suspension to be filtered. At this point the density of the uniform bacteria suspension is adjusted to approximately 75 x 1 of the MacFarland scale. The antigen is then centrifuged by means of a Sharples super-centrifuge.

The mass of bacteria is removed from the centrifuge bowl and resuspended in a solution containing one percent liquor formaldehyde, one percent monobasic potassium phosphate, and 0.85 sodium chloride. The volume of the suspending solution used should be approximately two-thirds the volume of the original centrifuged

solution. After the bacterial mass has been uniformly suspended in the above solution, it is again passed through a cotton-wool pad in a Buchner funnel without the aid of suction. The density of the suspension is determined, which at this point should be from 100 to 120 x 1 of the MacFarland scale, and the product is diluted with a sufficient amount of the diluent to produce a density of 75 x 1 of the MacFarland scale. In adding the required amount of diluent, allowance is made for the addition of a one percent aqueous solution of crystal violet in such proportion that the final product will contain 3 cc. of the one percent crystal violet solution in each 100 cc. The diluent is the phosphate mixture described on page 2. The required volumes of dye solution and diluent are mixed and then added to the resuspended bacteria. After mixing, the antigen is allowed to stand 48 hours before use. The average yield of finished antigen from 100 cc. of culture medium is approximately 50 to 60 cc.

6-4-57